Chihiro TAKAHASHI*: Studies on the regeneration of detached organs in pteridophytes (1) *Pteris vittata, Lygodium japonicum* and *Equisetum arvense*

高橋千裕*: 羊歯植物における切除器官の再生に関する研究 (1) モエジマシダ, カニクサおよびスギナ

It is very common in the plant kingdom that a part isolated from a whole plant has the ability to develop into a new whole plant. This was proved even in single cells when they were cultured under proper conditions (Steward, Mapes, and Mears 1958). Sporophytes of pteridophytes also have the ability to produce the regenerative outgrowth, naturally, of sporophytic nature. Besides this, they have the ability to produce the regenerative outgrowth of gametophytic nature, that is, the aposporous outgrowth. In this case they change the growth pattern from the sporophytic growth to the gametophytic growth without the intervention of sporogenesis. This apospory has provided a strong clue to the experimental study on the alternation of generations, one of the most fascinating but perplexing themes in plant morphology. Studies on apospory were reviewed by Steil 1939 and 1951.

The present writer has studied on apospory in pteridophytes from various aspects. From the obtained results he proposed a hypothesis about the mechanism responsible for the induction of apospory (Takahashi 1962). According to this the correlation among various organs maintains and fortifies the sporophytic nature in the young sporophytes and therefore, destroying this correlation results in the change of the sporophytic conditions into the gametophytic conditions in a given organ. This was proved by the fact that the growth pattern varied according to the organ combination isolated from a whole plant (Takahashi 1968). It appeared that investigations on the induction of regeneration including apospory in many pteridophytes were necessary in order to compare the various growth patterns of the outgrowth in different species, to find favourable species for detailed study, and to

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verify and advance the above hypothesis. The present study was carried out along this line. The induction of regeneration was attempted in three species of fern and horsetail. There had been no report on apospory in them.

The writer's thanks are due to Professor Emeritus Masao Kumazawa for his kind guidance and encouragement in the course of this study.

Material and methods

(1) Plant material Two species of Filicales, *Pteris vittata* L., *Lygodium japonicum* Sw., and one species of Equisetales, *Equisetum arvense* L., were used in this study. *Pteris vittata* was grown in a green house at Nagoya University, and *Lygodium japonicum* and *Equisetum arvense* were growing in the university campus.

(2) Culture of prothallia Many young sporophytes of these plants were sexually produced by prothallia starting from spores in the laboratory. Spores were collected from these plants at the time of maturation. Spores of *Pteris vittata* and *Lygodium japonicum* had been stored in a desiccator at the room temperature until they were sown. Spores of *Equisetum arvense* were sown just after the collection because the spore viability is of very short duration in this species.

Spores were sown in Petri-dishes of 6 cm in diameter, which contained the culture medium consisting of Knop's solution and 1 per cent agar. Knop's solution of the fivefold dilution was used for *Pteris vittata* and *Lygodium japonicum*, Knop's solution of the original concentration for *Equisetum arvense*. Petri-dishes were kept at 21°–27°C and under continuous white illumination of 700–1,500 luxes from fluorescent tube in an incubator. Under these conditions prothallia developed normally in all three species.

(3) Surgical operation of sporophytes The prothallia gave rise to young sporophytes with leaves and roots about one to three months after the spore sowing, which differs in different species.

Operations were made with a fine scalpel and forceps. In *Pteris vittata* and *Lygodium japonicum* the first, second and third leaves were detached from a whole sporophyte near the base of the stipe and the first roots near the base. In *Equisetum arvense* a shoot of the sporophyte was differentiated into nodes and had a whorl of scaly leaves at each node. Young sporophytes with the first shoot consisting of one to three nodes and the

first root were used. The shoot and the root were detached from a whole sporophyte near the base.

(4) Culture of detached organs 20 to 30 detached organs were cultured in each Petri-dish which contained Knop's solution of the fivefold dilution for *Pteris vittata* and *Lygodium japonicum*, and Knop's solution of the original concentration for *Equisetum arvense*. Petri-dishes were kept at 22°–27°C and under the natural dispersed light of 1,500 luxes in maximum in a glass-walled incubator.

Contaminations were more or less not good for cultures. Especially, good results were not obtained when cultures were seriously contaminated with algae. Such cultures were eliminated. Fungi contaminations in the culture solution were not so harmful because they usually disappeared soon. But dead leaves or roots were removed when or before they were molded. The culture solution was supplied when it decreased much for the evaporation in a long period of culture. The cultures had been observed under the microscope for two to three months.

Results

(1) *Pteris vittata* 1738 of the first, second and third unfolded leaves and 250 of the first roots were detached and cultured. The first, second and third leaves were cultured distinguishably in Petri-dishes different from each other. More than half of them were the first leaves. After three months most leaves had died without regeneration but 31 leaves gave rise to 32 regenerative outgrowths. No outgrowth occurred on detached roots.

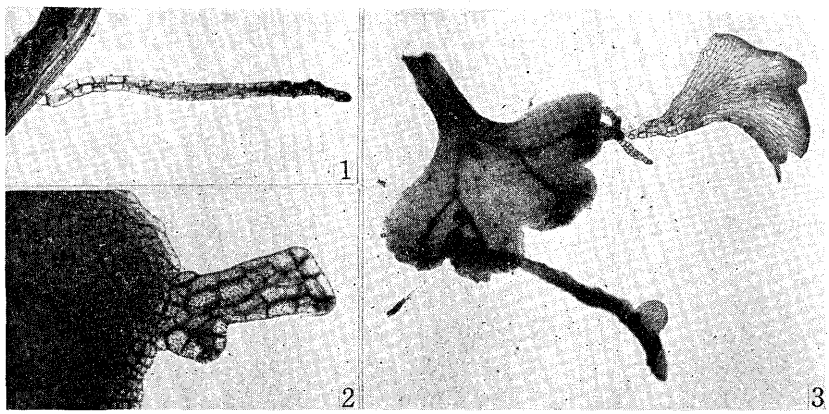
Tab. 1. Growth pattern of the outgrowth and part of leaves which gave the outgrowth in *Pteris vittata*.

Growth pattern	Leaf part		Sum
	Lamina	Stipe	
Gametophytic	6	5	11
Sporophytic	3	11*	14
Unidentifiable	1	6	7
Sum	10	22	32

* One case of the rachis origin was also included here.

The obtained results are shown in Table 1. Some of the outgrowths were recognized unquestionably as the sporophytic ones because they had stomata and/or vascular structure, and some as the gametophytic ones because they had gametangia and rhizoids. As for the others, however, it was impossible to identify the growth pattern, because they did not exhibit any morphological clue. This type of outgrowth will be designated as the unidentifiable outgrowth hereafter. In one case the leaf gave rise to both the gametophytic and the sporophytic outgrowths in different parts of the same leaf.

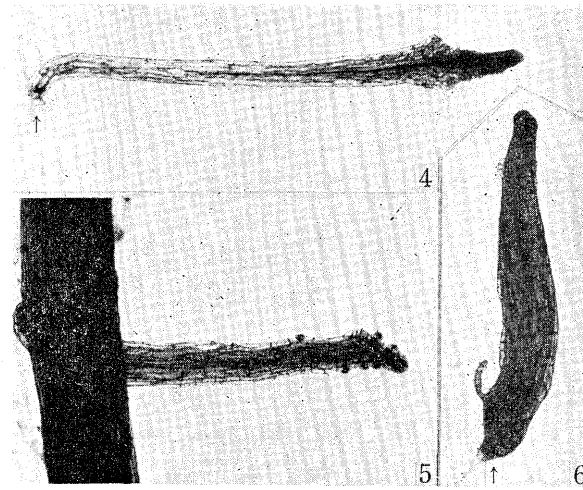
Most of the gametophytic outgrowths remained filamentous (fig. 1), and produced rhizoids and antheridia containing motile spermatozoids. The others became flat and thalloid (fig. 3), only one of which succeeded in producing archegonia by the end of this experiment. The occurrence of apospory was restricted to the first leaf. The writer failed to get the sporophyte sexually produced on such an archegonium-bearing aposporous prothallium.



Figs. 1-3. Gametophytic outgrowth of *Pteris vittata*. Fig. 1. Gametophytic outgrowth. It bore antheridia and originated from an epidermal cell of stipe. ×25. Fig. 2. Gametophytic outgrowth. It originated from marginal cells of lamina. ×20. Fig. 3. Heart-shaped and filamentous aposporous prothallia. They occurred on the same lamina. ×15.

The sporophytic outgrowths were formed more or less abnormally and failed to develop into the normal sporophyte. Some of these abnormal forms will be described here. Fig. 4 shows a leaf-like outgrowth consisting of a stipe-like cylindrical portion and a lamina-like flattened portion on which

stomata were distributed. The vascular structure running through inside the outgrowth was clearly observed but was not connected with that of the leaf which produced the outgrowth. This leaf-like organ was derived from one cell of the stipe epidermis. The cylinder-like outgrowths were often observed. One of them bore stomata at their distal portion and was derived from one cell of the stipe epidermis (fig. 5). Another outgrowth as shown in fig. 6, was derived from two cells of the stipe epidermis, had the vascular structure and was covered with several hairs. Its proximal portion was constructed out of cells that were large and not-oriented, while its distal portion was constructed out of cells that were small and oriented. The latter failed to grow unlimitedly though it seemed meristematic. This outgrowth happened to be separated from the leaf, when the photomicrograph was taken. It decayed soon. In all cases the vascular structure in outgrowths was not connected with that of the leaf which produced them.



Figs. 4-6. Sporophytic outgrowth of *Pteris vittata*. Fig. 4. Leaf-like outgrowth. Stomata and vascular structure were seen in this outgrowth. The arrow indicates the part where this outgrowth was attached to the stipe. ×35. Fig. 5. Cylinder-like outgrowth. Dark dots in the distal portion are stomata. ×35. Fig. 6. Sporophytic outgrowth. Hairs and vascular structure were seen. The arrow indicates the part where this outgrowth was attached to the stipe. ×35.

One of the unidentifiable outgrowths is shown in fig. 7. This outgrowths assumed neither prothalloid form nor sporophytic form in appearance, and had no sexual organ, rhizoid, vascular structure and stoma.

As for the part where the outgrowth took place, it was on every part of the lamina and the stipe. It appeared that there was a polarity in the occurrence of the sporophytic and the gametophytic outgrowths along the

leaf axis from the lamina to the stipe, that is, the gametophytic outgrowth tended to occur on the lamina and the sporophytic outgrowth on the stipe. Table 1 shows that three sporophytic outgrowths were brought about on the lamina. But they occurred in the basal part of it near the stipe.

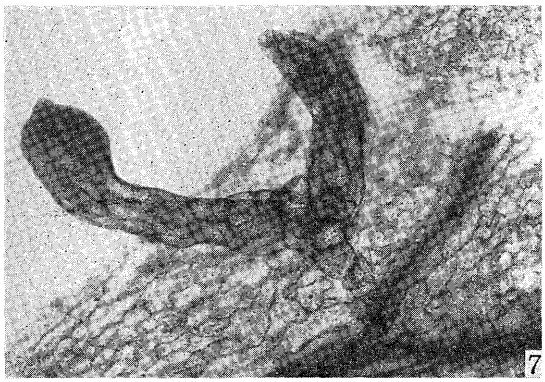


Fig. 7. Unidentifiable outgrowth of *Pteris vittata*. It originated from an epidermal cell of lamina. ×60.

Both the gametophytic and the sporophytic outgrowths were usually of the epidermal origin. It also appeared that there were those which originated from the inside tissue of the leaf, such as the mesophyll and the cortex of the stipe. The outgrowth originated from one to several sporophytic cells.

The sporophytic outgrowth was observed usually on the first leaf, rarely on the second leaf, and only in one case on the third leaf. The earlier leaves appeared to have the larger regenerative ability than the later leaves. On the other hand the gametophytic outgrowth was restricted to the first leaf as far as the present study was concerned.

The ability to produce the outgrowth was very small in this species, though both the sporophytic and the gametophytic outgrowths occurred. It has not been observed in this species that the *in situ* leaves on the intact plants showed the regenerative or adventitious growth.

(2) *Lygodium japonicum* 941 of the first unfolded leaves and 50 of the first roots were detached and cultured in this experiment. After three months it was observed that 7 of the leaves gave rise to the aposporous outgrowths. They developed into the cordate prothallia bearing antheridia but no archegonia. They originated from one cell of the lamina epidermis at or near the leaf margin. The leaves often showed the surplus growth at their margin especially near the terminal of the vein, but this was not

causally related with the regenerative process. The leaves did not show any regenerative outgrowth unless they were detached.

None of the roots gave rise to any outgrowth. However, the root number tested was too small to conclude that the root had no regenerative ability. No sporophytic outgrowth was observed. The ability to regenerate was very small in this species.

(3) *Equisetum arvense* The writer failed to induce the gametophytic regeneration by the method of the organ detachment as far as this experiment was concerned. When the first shoots of young sporophytes grew into those consisting of one to three nodes, the first shoots and roots were detached and cultured for two to three months. Because the leaves were small and fused into a sheath, the writer detached the shoots with or without the apex instead of detaching leaves.

More than half of 175 shoots with an apex grew further, formed new nodes successively, and gave new branches at their nodes sooner or later and adventitious roots too. The more the nodes of the detached shoots were, the greater the possibility of the survival of the shoots was. The survivors of the detached shoots were as follows; 23 of 45 shoots with one node, 22 of 32 shoots with two nodes, all of 8 shoots with three nodes.

131 detached shoots were decapitated, that is, they were deprived of the shoot apex. They developed new shoots at the node in the normal way but earlier than usual.

The writer observed only a few club-like outgrowths growing at the node, and was unable to decide the growth pattern. These outgrowths were made up of relatively large and unspecialized cells, were limited in the growth, and were related with neither the gametophytic nor the sporophytic regeneration. It was likely that these outgrowths resulted from the node injury at the time of operation. No roots exhibited any outgrowth in this experiment.

Discussion

Growth patterns in the regenerative outgrowth From the consideration of previous and present studies some growth patterns are distinguished in the regenerative outgrowth. They are as follows; (1) the sporophytic outgrowth, (2) the gametophytic outgrowth, (3) the intermediate outgrowth,

and (4) the unidentifiable outgrowth.

What growth pattern occurs, differs in different species. For example, only the gametophytic outgrowth occurred in *Pteridium aquilinum* (Bell and Richards 1958, Takahashi 1962) and *Lygodium japonicum*. In *Pteris vittata* as in the two *Asplenium* species (Morlang 1967) both the sporophytic and the gametophytic outgrowths occurred on the same leaf. The intermediate outgrowth which bears both the sporophytic and the gametophytic characters in the same outgrowth, occurred in some species (Goebel 1907, Beyerle 1932, Lawton 1936) but not in the present species.

There were such outgrowths as did not provide any strong clue to identify the growth pattern. In this report these outgrowths are designated as the unidentifiable outgrowth for convenience' sake. In this outgrowth there may be included (1) the callus which has remained undifferentiated by the time of observation and (2) the outgrowth which has not developed by then any characteristic form and structure in appearance, though the growth pattern had been determined. However, as a matter of fact, it is difficult to distinguish them. It seems that the unidentifiable outgrowth as observed in *Pteris vittata* is the latter. The detached leaves were able to produce the callus (Bristow 1962, Morel 1963). From the consideration of previous reports it seems that the induction of callus is easier when they are cultured on the organic medium than on the inorganic medium. *Pteridium* leaves produced the callus-like outgrowth when cultured in Knop's solution (Takahashi 1962). However, the callus-like growth was limited, the growth pattern soon changed, and the callus-like outgrowth, in turn, produced the gametophyte. This may result from the shortage of organic nutrition by the death of leaves. By contrast, on the organic medium the enough supply of organic nutrition makes the callus grow unlimitedly regardless of the death of leaf cells.

The ability to produce the regenerative outgrowth The ability to produce the regenerative outgrowth differs greatly in different species (Beyerle 1932). *Pteridium aquilinum* var. *latiusculum* produced more outgrowths both in the percentage of the leaves and per leaf than *Pteris vittata* and *Lygodium japonicum*. In *Cystopteris fragilis* 80 per cent of the leaves produced outgrowths (Lawton 1936). This is the first report on apospory in *Pteris vittata* and *Lygodium japonicum*. As for the former it was reported

that the leaves cultured on the organic medium gave rise to the callus, but not the aposporous outgrowth (Kato 1965). The present writer failed to induce apospory in *Equisetum arvense*. There has been no report of apospory in the Sphenopsida.

The ability to produce the regenerative outgrowth also differs in different organs in the same plant. Roots exhibit the very small regenerative ability. Probably it is especially difficult for roots to produce the sporophytic outgrowth. There has been only a report that it occurred in *Woodsia obtusa* (Lawton 1932). The gametophytic outgrowth rarely occurred on roots of *Pteridium aquilinum* var. *latiusculum* (Takahashi 1962), but not in *Pteris vittata* and *Lygodium japonicum*. It is likely difficult, for shoot apices and roots which include the meristem and are not the photosynthetic organ, to produce the aposporous outgrowth (Takahashi, 1968). On the contrary leaves which were the photosynthetic and not the meristematic organ, produced the aposporous outgrowth far more easily. If the same is true for *Equisetum arvense*, the decapitation of the shoot may not have been enough for inducing apospory, because the node may act as the meristem and develop a new shoot. However, to culture only the internode excluding the node, was not attempted in this study, because the object was too small to operate surgically.

It seems that what growth pattern occurs, differs in different parts of a leaf, as shown in *Pteris vittata*. The regional difference of internal conditions in a leaf may be responsible for the polarity that is expressed by the different growth pattern of the regenerative outgrowth. Further study for clarifying these conditions is needed and is being planned.

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3 種の羊歯植物の若い胞子体において，実験的な再生の誘起を試みた。モエジマシダとカニクサでは葉と根を，スギナでは苗条と根を，それぞれ切り取り，クノップ液で培養したところ，次の結果を得た。モエジマシダでは，少数の葉が再生体を生じた。この再生体には，胞子体の特徴を示すもの，配偶体の特徴を示すもの，これらいずれの特徴も示さないもの，が区別された。胞子体的再生体は葉柄に，配偶体的再生体は葉身に，それぞれ多く生じる傾向があり，極性が関係するらしい。カニクサでは少数の葉が配偶体的再生体のみを生じた。スギナの苗条では茎端で正常に成長する。茎端を除去すると節から新しい苗条を生じるが，これは無処理のものに較べ枝分れが早いに過ぎない。根においては 3 種ともいかなる再生も認められなかった。

○ニイタカアカマツの腹こう細胞核の分裂（杉原美徳） Yosinori SUGIHARA: The mitosis of the ventral canal nucleus in *Pinus taiwanensis* Hayata

マツ属植物の蔵卵器では受精のおこるすこし前に雌性中心細胞の核が分裂して卵核と腹こう細胞核とができる。その 2 核の間に細胞壁が形成されて大きな卵細胞と小さい腹こう細胞とになる。1936 年に田原正人（科学 6: 462, '36）はアカマツ（*Pinus densiflora* Sieb. et Zucc.）で腹こう細胞の核が分裂像を示す場合を報告している。筆者は 1940 年 7 月に台湾の新太平山（当時の呼称）で採集したニイタカアカマツで分裂中期の像を観察した (Fig. 1)。染色体数は n=12 で，Saylor (Sylvae Genetica 13: 165-192, '64) が根端で 2n=24 と報告しているものと一致する。このような分裂により生じたと思はれる 2 核性の腹こう細胞や，腹こう細胞が 2 個になっている場合はみられなかった。マツ属でみられるこのような分裂像がはたしてどのような意味をもつかは明らかでない。ただ羊歯植物で腹こう細胞核が 2 個以上になる場合は西田・佐久間（植研 36: 142-152, '61）によると *Todea* (Stokey & Atkinson '56) ではまれに 3 細胞になり，*Cibotium* (Stokey '30) ではしばしば 2 細胞になり，又 *Blechnum* (Stokey & Atkinson '52) や *Dipteris* (Stokey '45) ではときとして 2 細胞になっているということである。しかしこれ等のことと田原 ('36) のアカマツや

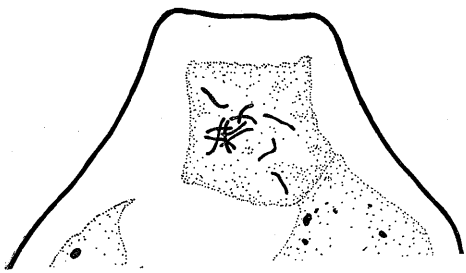

Fig. 1. Mitosis in ventral canal nucleus in *Pinus taiwanensis* Hayata. ×440.